One ISH, two ISH, red ISH, blue ISH: choosing the right in situ hybridisation protocol

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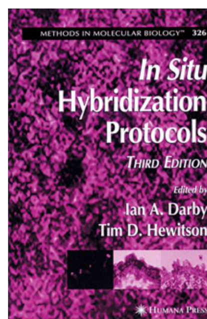

In Situ Hybridization Protocols, Third Edn

Edited by Ian A. Darby and Tim D. Hewitson

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It is nearly a quarter of a century since the groundbreaking experiments of Ernst Hafen (Hafen et al., 1983) and Mike Levine (Levine et al., 1983), who first applied the technique of in situ hybridisation (ISH) to embryo specimens. These experiments provided the first insights into the spatial distribution patterns of specific transcripts in different regions of the embryo. Since then, the technique of mRNA ISH has become central to the study of developmental biology and is routinely used to assess the spatiotemporal expression pattern of newly identified genes, which is a necessary first step towards understanding the roles of such genes during embryogenesis. More recently, other techniques, such as microarray-based expression profiling, have been increasingly used to assess gene expression patterns on a genomic scale from dissected or micro-dissected embryonic material. However, as these methods do not yield spatial information about a gene's expression pattern, ISH is now often used to validate such data. When used in this context, ISH helps to refine our understanding of the spatiotemporal expression patterns of molecules that have been identified by high-throughput methods.

Despite being a commonly employed technique, ISH still retains its reputation of being notoriously difficult to perform. For the novice especially, it can be a daunting prospect to trouble-shoot if, at the end of several days of diligently following a protocol, applying and removing one reagent after another, either no staining pattern is obvious or background staining obscures all. Was it the preparation of the specimen that was the problem? Was there RNase contamination of a reagent? Perhaps the choice of probe was unsuitable? Maybe the hybridisation temperature was too high or too low? Because of the relatively complex nature of the technique, the list of problems to troubleshoot can appear to be endless. As such, no one embarking on experiments that require the use of ISH should be without at least one manual that contains various methods for this technique. In addition to the technical difficulties that can be encountered with ISH, it is also a method that is continuing to develop with recent advances in new probe chemistries, non-radioactive detection methods, signal amplification and the development of derivative techniques. Thus, even those experienced in the art of ISH need to keep up-to-date with these exciting advances in order to apply them to areas of their own research. In these regards, the third edition of *In Situ Hybridization Protocols* is a book that goes someway to satisfying both the novice and the expert user.

In Situ Hybridization Protocols is divided into 19 chapters, each of which constitutes one particular protocol. As with all volumes in the *Methods in Molecular Biology* series, each chapter is formatted into five sections: Introduction, Materials, Methods, Notes and References. This allows the user to easily and quickly assess from the Introduction whether the protocol is suitable for their requirements and, if so, which reagents will be required. The Methods sections all offer step-wise protocols, which are clear and simple to follow, and the associated indexed Notes sections offer many helpful insights with relevant background theory and useful practical tips from the expert authors.

The main focus of this third edition is performing ISH to detect mRNA on sectioned material. (Note that if you are after a resource that offers multiple detailed methods to detect DNA in situ, this is not the book for you.) Chapter 1 begins with a simple protocol for the preparation of either frozen or paraffin-embedded tissue sections for ISH by microwave oven treatment. The authors state that this novel approach is useful for denaturing target mRNA to allow easier probe access, replacing or enhancing the protease digestion of frozen or paraffin-sectioned samples, as well as denaturing endogenous phosphatases, thus lowering background signal.

The next two chapters cover the preparation of the other crucial reagent of the ISH experiment – the probe. Chapter 2 describes methods to prepare a DNA template fragment and to radioactively or non-radioactively label a DNA probe, whereas Chapter 3 summarises methods for generating complementary (c)RNA ISH probes, and provides protocols for both isotopic and non-isotopic labelling. Also included in Chapter 3 are complete protocols for mRNA ISH on sectioned material. These protocols cover specimen preparation and embedding, hybridisation and detection methods, including the amplification of signal using biotinylated tyramide chemistry. As tyramide signal amplification is perhaps the most significant advance in recent years for increasing the sensitivity of non-radioactive ISH, the next chapter is devoted entirely to the theory and practice of this technique. Protocols are given to apply the method to both DNA ISH and mRNA ISH on a variety of sample types, such as cell preparations and paraffin-embedded tissue sections.

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The next three chapters are perhaps the most relevant ones for the readers of *Development*, as these list some tried-and-tested protocols specifically for assessing mRNA distribution by ISH in embryo specimens. The first of these chapters outlines protocols for assessing mRNA distribution in mouse embryos. Included are methods for cRNA probe synthesis (both radioactively and digoxigenin labelled), ISH on paraffin-embedded and frozen sections, and a method to perform whole-mount ISH. Associated with this excellent chapter are many notes that detail handy tips, covering everything from how to choose a suitable probe to lowering non-specific background in whole-mount samples. The second of this

group of chapters gives alternative protocols for carrying out non-radioactive ISH on both frozen sections and whole-mount mouse embryo samples, whereas the third chapter deals specifically with a method of ISH for whole-mount mouse embryos.

Most of the remaining chapters include other specialised and less-widely performed, yet potentially very useful, protocols. Chapter 10 details a method that combines electron microscopy and ISH, which allows ultra-structural examination of gene expression in situ. Chapter 11 outlines a protocol for ISH on free-floating sections (as opposed to the traditional approach of adhering the sections to a microscope slide), whereas Chapter 12 presents a protocol to detect low-abundance transcripts in adherent cultured cells. Chapter 13 discusses a protocol to identify xenotransplanted cells by DNA ISH (in this case, human cells transplanted into the mouse, identified by using a probe specific to a primate-specific repeat element), and Chapter 14 lists a set of protocols to perform DNA ISH on chromosome and extended fibre preparations from plant material, as well as mRNA ISH on paraffin-embedded plant specimens. Chapter 15 outlines a method to perform ISH to detect histone mRNA in order to identify cells in S-phase on paraffin-embedded sectioned material. This method offers an alternative to pulse-labelling samples with bromodeoxyuridine (BrdU) and can be applied to samples where BrdU labelling cannot be performed, such as human samples or archived sectioned material.

The book also covers methods for quantitatively and semi-quantitatively assessing differences in gene expression using ISH. The first of these (Chapter 18) covers a protocol for performing semi-quantitative ISH using radioactive probes. Details of probe and specimen preparation, hybridisation parameters and quantification of the data using image analysis software are discussed. The final chapter of the book gives a method that has been used to perform quantitative ISH on formalin-fixed, paraffin-embedded tissue microarrays using a phosphoimager.

As can be seen from these chapters, this book contains both a set of relatively standard mRNA ISH protocols, which will be of interest to the first-time user, and an impressive number of lesser-known applications of ISH, which will be useful to many developmental biology labs. This provision of a variety of ISH protocols – written independently by separate expert authors with an abundance of useful notes and helpful tips – is a distinct advantage of the book; however, one unfortunate drawback of this approach is that continuity between the chapters is somewhat lacking. For example, information is often repeated between chapters (such as methods of in vitro transcription for generating riboprobes) and is not cross-referenced, making it difficult to compare and contrast the methods. The book would also benefit from a general introductory chapter to outline the theory of ISH and give a comprehensive grounding of the technique for the first-time user. This would be very useful should trouble-shooting be required. It could discuss, for example, features to include or avoid when designing a fragment for use as a probe, the theory of how different tissue treatments can affect probe accessibility in a sample, and the pros and cons of radio-labelled probes versus non-radiolabelled probes. Most of this information is present in the book, but it can be rather difficult to find and can be hidden in a chapter that is not of immediate relevance to the reader. It is also a pity that all three chapters devoted to ISH on embryos focus on the mouse. Although each chapter mentions that the protocols can be modified for use in other organisms, suggestions as to how this can be achieved are not given and could thus prove challenging for the novice, especially without another protocol to compare to and contrast with.

Despite these drawbacks, this book does contain a lot of useful information, drawn from many experts in the field. As such, it would be a useful addition to the ISH methods already present on the lab bookshelf.

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Basic recipes for the molecular biologist

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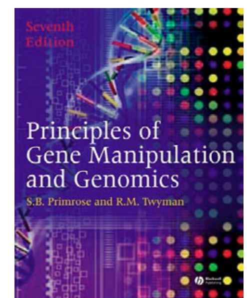

Principles of Gene Manipulation and Genomics, Seventh Edn

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"When *I* was a student I had to make my own *Eco*RI" my PhD supervisor would declare, in a manner akin to the gents in the four Yorkshiremen sketch that was popularised by Monty Python, as I reached into the freezer for a bit of the said enzyme. Skip forward two decades and my own graduate students are treated to similar reminiscences regarding the re-distilling of phenol or the making of sequencing gels, particularly when they are clutching requisitions for some molecular biology 'kit' that need my authorisation. Are these trips down memory lane simply the irrelevant ravings of grumpy middle-aged men or is there a point to these 'when I were a lad' stories? Molecular biology is a bit of an art; I mean art in its broadest sense of course, much in the same way that cooking is an art. After all, almost anyone can follow a recipe, but turning out a gourmet meal is a different matter, requiring an understanding of the individual ingredients and how they blend together to provide a result greater than the sum of the parts. Similarly with molecular biology: you can follow a protocol, but will that genomic library contain all the sequences you want (tasty) or be a collection of useless scrambled clones because you didn't do the partial digests properly (unpalatable). Thus, good molecular biology is built upon a sound understanding of the underlying biochemistry. Obviously, we old hands like

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